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A comparison of heart rate variability, *n-3* PUFA status and lipid mediator profile in age- and body mass index-matched middle-aged vegans and omnivores.

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Shortened Title: Heart rate variability and lipid mediators in vegans

Key words: vegan, *n-3* PUFA, *n*-6 PUFA, coronary heart disease, heart rate variability, cardiac autonomic function, inflammation, eicosanoid, prostaglandin, lipidomics, lipid mediator, oxylipins

ABBREVIATIONS

Alpha-linolenic acid, ALA; coronary heart disease, CHD; docosahexaenoic acid, DHA; eicosapentaenoic acid, EPA; heart rate, HR; heart rate variability, HRV; high frequency power, HF; low frequency power, LF; neuroprotection D1, NPD1; percentage of adjacent NN intervals that differ by > 50%, pNN50; polyunsaturated fatty acids, PUFA; root of the mean of the sum of the squares of differences between adjacent NN intervals, RMSSD; standard deviation of the average 5-min NN intervals, SDANN; standard deviation of normal-to-normal intervals, SDNN; very low frequency power, VLF

Abstract

Low heart rate variability (HRV) predicts sudden cardiac death. Long chain (LC) *n*-3 PUFA (C20-C22) status is positively associated with HRV. This cross-sectional study investigated whether vegans aged 40-70 y (*n* 23), whose diets are naturally free from eicosapentaenoic acid (EPA; 20 : 5*n*-3) and docosahexaenoic acid (DHA; 22 : 6*n*-3), have lower HRV compared to omnivores (*n* 24). Proportions of LC *n*-3 PUFA in erythrocyte membranes, plasma fatty acids, and concentrations of plasma LC *n*-3 PUFA-derived lipid mediators were significantly lower in vegans. Day-time interbeat intervals (IBI), adjusted for physical activity, age, BMI and sex, were significantly shorter in vegans compared to omnivores: mean difference -67 ms (95% CI -130, -3.4), P <0.05, but there were no significant differences over 24 h or during sleep. Vegans had higher overall HRV, measured as 24 h SDNN (mean adjusted difference 27 msec, 95% CI 1, 52, P = 0.039). Conversely, vegans presented with decreased 8 h day-time HRV: mean adjusted difference in SDNN -20 msec (95% CI -37, -3), P = 0.021, with no differences during nocturnal sleep. Day-time parameters of beat-to-beat HRV (RMSSD, PNN50% and HF) were similarly lower in vegans, with no differences during sleep. In conclusion, vegans have higher 24 h SDNN, but lower day-time HRV and shorter day-time IBI relative to comparable omnivores. Vegans may have reduced availability of precursor markers for proresolving lipid mediators; it remains to be determined whether there is a direct link with impaired cardiac function in populations with low omega-3 status.

INTRODUCTION

The longer chain (LC) *n*-3 PUFA (C20-C22), eicosapentaenoic acid (EPA; 20 : 5*n*-3) and docosahexaenoic acid (DHA; 22 : 6*n*-3), are mainly derived from seafood, although small amounts are provided by meat, eggs and dairy products. Consequently, vegans consume a diet devoid of 20 : 5*n*-3 and 22 : 6*n*-3 [(1)]. The main *n-3* PUFA in vegan diets is α-linolenic acid (ALA; 18 : 3*n*-3), derived from plant foods, particularly soya and seed oils such as rapeseed oil. (LC) *n*-3 PUFA as percentages of total fatty acids in blood fractions are, in vegans, only a third of the level in meat- and fish-eaters [(2)]. 20 : 5*n*-3 and 22 : 6*n*-3 can be endogenously synthesised from 18 : 3*n*-3 by desaturation and elongation enzymes, but the rate of this conversion is restricted to a narrow range in adults [(3)]. Stable isotope studies have suggested that conversion of 18 : 3*n*-3 to 22 : 6*n*-3 can vary from undetectable amounts up to 4% in men, and 9% in women [(4)]. An observational study suggested that conversion from dietary 18 : 3*n*-3 to longer chain *n*-3 PUFA might be increased in non-fish eaters[(5)]. Furthermore, dietary supplementation of vegans with 18 : 3*n*-3 has not been found to increase 22 : 6*n*-3 in blood lipids, including plasma choline phosphoglycerides and platelet phosphoglycerides [(6; 7)] and erythrocytes, platelets, and plasma cholesteryl esters, phospholipids and triglycerides [(6; 7)]. It is unclear whether the lack of 20 : 5*n*-3 and 22 : 6*n*-3 intake in vegans has adverse effects on cardiovascular health[(8; 9)] especially as body mass index[(10; 11)], blood cholesterol[(12; 13)] and blood pressure[(14; 15)] are lower than in meat-eaters.

LC *n-3* PUFA, especially 22 : 6*n*-3, are rapidly incorporated into cellular lipids, primarily membrane phospholipids, in a variety of cells including cardiomyocytes and neural tissue, thereby influencing membrane properties and function of membrane proteins. Fish oil consumption reduces HR in humans[(16)]. Increasing LC *n*-3 PUFA content in cardiomyocyte membranes by 3-wk of dietary fish oil in rabbits decreases HR in isolated hearts, and reduces pacemaker activity and pacemaker current in sinoatrial node cells[(17)]; mechanisms are likely to be related to increased membrane fluidity and direct interaction with a hyperpolarisation-activated I_f channel protein[(17)], altering ion channel currents and reducing intrinsic pacemaker rate, reviewed by Billman[(18)].

Raised heart rates are associated with a high degree of sympathetic activity and suppressed parasympathetic activity (vagal activity slows heart rate) resulting in low HRV; a reduced capacity to self-regulate the heart rate in response to physiological demands. Low HRV is

associated with mortality after a myocardial infarction [19; 20; 21], risk of sudden death in patients with coronary heart disease [22], and risk of cardiac events in the general population [23]. Higher *n-3* PUFA tissue status or fish consumption has been positively associated with HRV [24; 25]. Since HRV is under the control of the autonomic nervous system, regulation of HR may be influenced by n-3 PUFA status of neuronal and cardiac tissue. The brain is particularly rich in 22 : 6*n*-3, and incorporation of dietary LC *n*-3 PUFA into neuronal tissue influences gene expression, membrane protein signalling, neurotransmission and signal transduction pathways[26]. This may influence autonomic function by enhancing parasympathetic and/or reducing sympathetic activity, thus reducing HR and increasing HRV. Therefore, impairment of cardiac autonomic function due to depleted LC *n*-3 PUFA content in the central or peripheral nervous tissue would reduce the responsivity of the heart.

A further mechanism whereby cardiac function may be modulated by neuronal LC *n*-3 PUFA status is via the production of eicosanoids and related PUFA-derived lipid mediators that may reduce inflammation and terminate ("resolve") acute inflammatory events, preventing further neuronal tissue damage. PUFAs can be oxygenated into numerous bioactive lipid mediators [27], and some of the 20 : 5*n*-3 and 22 : 6*n*-3 derived species act as precursors of the specialised pro-resolving lipid mediators (SPMs), resolvins, protectins and maresin, which are autocoid substances actively involved in the resolution of local inflammation[27; 28; 29]. Neuroprotectin D-1 (NPD1) is a neuroprotective lipid mediator derived from 22 : 6*n*-3 which might be particularly relevant to the preservation of optimal cardiac autonomic function[30].

This study aims to compare HRV between vegans and age/sex/BMI-matched omnivores, representing populations with low and adequate tissue *n-3* PUFA status, respectively. The primary hypothesis of the study is that vegans have higher HR/shorter interbeat intervals (IBI) and lower HRV compared to omnivores. Exploratory analysis of plasma 20 : 5*n*-3 and 22 : 6*n*-3 derived lipid mediator concentrations was conducted in order to provide mechanistic hypothesis-generating data that may help explain differences in HR/IBI/HRV between low and high LC *n*-3 PUFA status groups.

Materials and Methods

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the research ethics committee of King's College London (BDM/12/13-84). Written informed consent was

obtained from each subject. Twenty-three healthy, non-smoking men and women, aged 40-70 y who had been following a vegan diet for at least 2 years were compared with 24 age- and BMI-matched healthy participants who followed a mixed diet including meat, fish, eggs and dairy-containing foods (omnivores). Primary outcome variables were HR/IBI and time domain parameters of different components of HRV: SDNN (standard deviation of normal-to-normal intervals: the most commonly reported marker of HRV and an indication of overall HRV, mainly determined by day/night differences) and RMSSD (square root of the mean of the sum of the squares of differences between adjacent NN intervals; an indicator of beat-to-beat, respiration-driven variability representing parasympathetic cardiac regulation). Secondary outcome variables included: other time and frequency domain and non-linear parameters of HRV, erythrocyte and plasma fatty acid composition, plasma oxygenated lipid mediator profile, fasting plasma lipid profile, vitamin B12, serum 25-hydroxyvitamin D, interleukin-6 (IL-6), fasting plasma glucose, blood pressure, body composition and background diet in order to compare risk factors for CVD in vegans and omnivores. A sample size of 23 in each group has a 80% power to detect a difference between SDNN means of 25 ms and between RMSSD means of 15 ms with a significance level of 0.05 (two-tailed), based on SDs of 30 ms and 18 ms respectively obtained from sleep-time HRV recordings in a previous cohort of middle-aged to older healthy men and women[31]. Participants were recruited by distributing adverts to vegan organisations and societies. Omnivore participants were recruited through internal and external email circulars and posters amongst university students and staff. The study was also promoted via social media, flyer distributions to vegan restaurants and vegan food shops throughout London, and at various vegan food events. Volunteers who responded to advertisements were given more information about the study, completed an initial eligibility questionnaire via telephone or e-mail and, if eligible, were provided with a study information sheet. Exclusion criteria included a reported history of CVD, diabetes, cancer (excluding basal cell carcinoma) in the past five years, chronic renal, liver or inflammatory bowel disease, history of drug or alcohol abuse (previous weekly alcohol intake >60 units/men or 50 units/women), current self-reported weekly alcohol intake exceeding 28 units, current use of marine *n-3* supplements, pregnancy, weight change of more than 3 kg in the previous 2 months, and BMI <18.5 and >35 kg/m2. Vegan subjects were enrolled on the study along with omnivore controls, aiming to match for sex, age (±5 y) and BMI (±2 kg/m^2). A validated food frequency questionnaire[32] was used to verify self-classification of dietary status of eligible volunteers and to provide supplementary

information on habitual dietary intakes. Analysis was carried out using an Excel spreadsheet that incorporated additional food composition data on LC *n*-3 PUFA contents of foods other than fish (meat, dairy, eggs).

Participants attended one study visit, which took place in the morning. Volunteers were instructed to fast for 12 h before the visit and consume nothing but water until attending the clinic. Once written informed consent was obtained, seated blood pressure was measured in triplicate using an A&D Medical UA-767Plus (San Jose, USA) upper arm automatic blood pressure monitor, in accordance with guidelines from the British Hypertension Society (BHS). Height, body weight and percentage body fat, and waist circumference (WC) were measured using a stadiometer, a Tanita weighing scale (Tanita UK Ltd, model: BC-418 MA; Middlesex, UK) and a tape measure, respectively. Participants completed the food frequency questionnaire, which was checked for completeness and any missing data verified directly with the participant. Fasting plasma glucose and serum lipids, serum liver function markers, and whole blood haematology was analysed on the same day in fresh blood samples, and further plasma aliquots were frozen at -70 °C until analysis of fatty acid and lipid mediator profiles could take place. Erythrocytes were washed with saline and lysed. The erythrocyte lysate was de-proteinised in the presence of butylated hydroxytoluene, chloroform was added to extract lipids then centrifuged as previously described [33]; supernatant was frozen at -20 ºC until analysis for fatty acid composition could be conducted [33]. An Actiheart monitor was fitted on the chest (CamNtech Ltd, Cambridge, UK), which they wore for 24 h. A diary was provided during the recording period to keep a register of all the daily activities (activities/exercise, meals or naps).

Heart rate variability measurements

IBI and continuous HR were measured for approximately 24 h using Actiheart monitors (Camntech Ltd, Cambridge, UK), which are small, light-weight (<10g) waterproof devices that also contain piezoelectric sensors to record acceleration in the vertical plane (counts per minute, cpm) as a measure of physical activity [34]. Before the monitor could be fitted, the area of skin was prepared including shaving of chest hair where required, using alcohol wipes to clean and dry the skin and use of an abrasive pad (UnilectTM) to remove the top layer of skin cells. Two ECG electrodes (SP-50, 50mm round, Pulse Medical) were placed on the chest to fit the Actiheart monitor. A short signal test involving a 5 minute walk was

performed before programming for the 24h recording to confirm adequate signal-to-noise ratio. Data processing of the 24 h IBI recordings was carried out using the Actiheart software (version 4.0.91, CamNtech Ltd, Cambridge, UK) and Kubios HRV analysis software (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Finland)[35]. Heart rate variability (HRV), HR/IBI and accelerometry data[34] were analysed for the full length of recording time (minimum of 18 h, up to 24 h). Further analysis was carried on a standardised day-time period of 8 h and sleep-time period of 2 h to remove the influence of variability in recording duration on HRV parameters. HRV outcomes included time and frequency domain parameters; time domain parameters are based on the time intervals between adjacent QRS complexes (normal-to-normal (NN) intervals) whereas frequency domain parameters employ power spectral analysis of NN intervals to determine the power (variance) within frequency bands [36]. Time domain parameters included SDNN, standard deviation of the average NN intervals in 5 min segments of the whole recording (SDANN), RMSSD, the percentage of adjacent NN intervals that differed by more than 50% (pNN50), and triangular index (Ti), the integral of the density distribution (the number of all NN intervals) divided by the maximum of the density distribution. Frequency domain parameters included high frequency (HF), low frequency (LF), and very low frequency (VLF) power, and the ratio of the LF and HF frequency band powers (LF/HF). A non-linear parameter using Poincaré plots of short term variability (SD1) against long term variability (SD2) was also calculated as a measure of complexity of HRV distribution over the duration of the recording. SDNN, LF and Ti represent overall variability. Short-term (beat-to-beat) components of HRV include RMSSD, pNN50, and HF. SDANN and VLF reflect longer-phase components of variability.

Fatty acid analysis

Proportions of fatty acids in whole plasma and erythrocyte membranes were analysed by GC (Agilent 7890A GC; Agilent Technologies) with a BPX70 GC column (length 25 m, internal diameter 0.32 mm, film thickness 0.25 μm) custom designed for separation of fatty acid methyl esters (SGE Analytic Science) following transesterification, as previously described[37], but substituting toluene for benzene and using pentadecanoic acid as an internal standard. Since total plasma concentrations of fatty acids differ in vegans compared to omnivores, individual plasma fatty acids were compared between groups as weight

178 percentages of the sum of fatty acids (% weight) [38] The omega-3 index was defined as the
179 sum of % weight EPA+DHA in erythrocytes.

180 Mediator Lipidomics

181 Prostanoids and hydroxy fatty acids derived from dihomo-gamma-linolenic acid (DGLA; 20 :
182 3*n*-6), linoleic acid (LA; 18 : 2*n*-6), 18 : 3*n*-3, arachidonic acid (AA; 20 : 4*n*-6), 20 : 5*n*-3 and
183 22 : 6*n*-3 were extracted from plasma and analysed by ultraperformance liquid
184 chromatography with electrospray ionization and tandem mass spectrometry (LC/ESI-
185 MS/MS) as previously described [39]. Briefly, samples were extracted in 15 % (v/v)
186 methanol, and internal standards were added (20 ng each of PGB_2-*d*4, 12-HETE-*d*8, 8,9-
187 EET-*d*11 and 8(9)-DHET-*d*11. Lipid extracts were semi-purified using solid phase extraction
188 (C18-E cartridges; 500 mg, 6 ml; Phenomenex, Macclesfield, UK), and dried under nitrogen,
189 before reconstitution in ethanol for analysis. Chromatographic separation was performed on a
190 C18 column (Acquity UPLC BEH, 1.7 µm, 2.1 x 50 mm; Waters) using a gradient of
191 acidified acetonitrile and water (Acquity Ultraperformance Liquid Chromatography and
192 Xevo triple quadrupole mass spectrometer, Water, UK). Analytes were recorded using
193 multiple reaction monitoring (MRM) assays, using the transitions reported in Astarita *et al.*
194 [27] and quantified using calibration lines constructed with commercially available standards
195 (Cayman, USA).

196 Blood biochemistry analysis

197 Blood samples were collected into fluoride oxalate tubes for glucose analysis and SST™ II
198 tubes for TAG, total cholesterol, and HDL cholesterol, vitamin B12, 25-hydroxy vitamin D
199 and interleukin-6 (IL-6) analysis; plasma and serum were stored frozen at -40 °C until
200 analysis (Becton Dickinson, UK). Analyses of full blood counts, plasma glucose, serum
201 lipids, vitamins, and IL-6 were determined by a Clinical Pathology Accredited (CPA)
202 Clinical Biochemistry laboratory (ViaPath, Kings College Hospital, London). Glucose and
203 lipids were analysed following enzymatic methods using reagents supplied by Bayer
204 Diagnostics Europe Ltd (Bayer House, Strawberry Hill, Newbery, Berks) using an ADVIA
205 2400 analyser (Sieman's Healthcare Diagnostics, Frimley, Surrey, UK). IL-6 was analysed
206 using a high sensitivity cytokine chip array assay (Human cytokine HS X biochip, Randox
207 Laboratories Limited, County Antrim). Serum vitamin D and B12 concentrations were

208 analysed using the ADVIA Centaur total Vitamin D and Vitamin B12 immunoassays
209 (Siemens Healthcare Diagnostics Ltd, Frimley, Surrey, UK).

210 Statistical analysis

211 Statistical analyses were performed using IBM SPSS Statistics 21.0 (Statistical Product and
212 Service Solutions, IBM Corp). Chi-squared tests for categorical variables and independent
213 samples t-test for continuous variables were used to assess the differences between vegan and
214 omnivore subjects' characteristics, dietary intakes, erythrocyte and plasma fatty acids, and
215 lipid mediators. Non-normally distributed data were normalised by natural logarithm (LN)
216 (results shown as geometric means with 95% CI) before analysis by independent t-test. If LN
217 transformation failed to yield a normal distribution, a Mann-Whitney U test was applied to
218 compare groups (results shown as medians with lower and upper quartiles). In the case of
219 lipid mediators, results from non-normally distributed data analysed by Mann-Whitney U test
220 were shown as medians with minimum and maximum values due to the proportion of
221 undetectable concentrations of LC n-3 PUFA-derived mediators in omnivores as well as
222 vegans.

223 For HRV analysis, normally distributed raw data or LN transformed data were analysed by
224 univariate ANCOVA, adjusted for sex, age, BMI and, in the case of day-time and 24 h data,
225 physical activity (accelerometry data). Results are expressed as estimated marginal means
226 (95% CI), adjusted for gender, age, BMI and 24 h activity for 24 h HRV and sleep-time –
227 day-time HRV, or gender, age, BMI and 8 h activity for 8 h day-time HRV, or adjusted for
228 gender, age and BMI only for 2 h sleep-time. Estimated marginal means and 95% CI from
229 data that were LN transformed before analysis by ANCOVA were back-transformed and
230 expressed as geometric means (95% CI). Data that could not be normalised by LN
231 transformation were analysed using Mann-Whitney U test and significance values are
232 presented unadjusted, with results shown as medians (lower and upper quartiles).

233 Results

234 Participant characteristics

235 **Figure 1** shows the flow of participants through the study. Subject characteristics of the 47
236 participants who completed the study are presented in **Table 1**. The mean ages of vegans (8
237 men, 15 women) and omnivores (12 men, 12 women) were 49 y (SD 8) and 54 y (SD 9)

respectively, and there were no significant differences in mean age or BMI, or distributions of sex between groups, although the sex distributions were not fully balanced across groups. Furthermore there were no significant differences in other markers of body composition (% body fat, waist circumference). Seated resting heart rate was on average 7 bpm higher and systolic blood pressure was 7 mm Hg lower in vegans compared to omnivores; there was no difference in mean seated diastolic blood pressure (Table 1). Fasting serum total and LN serum LDL cholesterol concentrations were lower in vegans compared to omnivores, but there were no differences in mean fasting plasma glucose, LN serum triacylglycerol, serum HDL cholesterol, serum vitamin B12, 25-hydroxy vitamin D or interleukin-6 concentrations, nor blood haemoglobin concentrations, indicating that the vegan group did not differ in vitamin D status and were likely to be taking dietary vitamin B12 supplements. Analysis of food frequency questionnaires (Table 1) showed that 61% of vegans reported taking vitamin B12 supplements, and suggested that vegans and omnivores had comparable total energy and percentage energy as fat intakes. Omnivores reported significantly higher protein (%E), saturated fat (%E) and food-derived vitamin B12 (µg) intakes and vegans had significantly higher carbohydrate (%E), total PUFA (%E), and 18 : 2*n*-6 (g) intakes. There were no differences in reported 18 : 3n-3 (g) intakes. As expected, vegans reported no dietary intake of 20 : 5*n*-3 and 22 : 6*n*-3, hence omnivores obtained significantly higher intake of these fatty acids, with estimated median intakes (IQR) of 0.14 g/d (0.09, 0.24) and 0.45 g/d (0.30, 0.81) for 20 : 5*n*-3 and 22 : 6*n*-3 respectively. A subset (12 omnivore and 8 vegan participants) completed 4-day food diaries (data not shown); analyses of these supported the food frequency questionnaire data.

Fatty acid and lipid mediator profiles

Vegans had a significantly higher proportion of plasma and erythrocyte 18 : 2*n*-6, plasma 18 : 3*n*-3 and erythrocyte 20:3 *n*-6 compared to omnivores (**Table 2**). Both whole plasma and erythrocyte membrane proportions of 20 : 5*n*-3 and 22 : 6*n*-3, plasma palmitic acid (16 : 0), and erythrocyte docosapentaenoic acid *n*-3 (22 : 5*n*-3) and palmitoleic acid (16 : 1*n*-7) were significantly lower in vegans compared to omnivores. Vegans had a significantly lower omega-3 index, with a geometric mean of 2.7% compared to 5.4% in omnivores, although both groups would be considered below the proposed omega-3 index cut-off of >8% for optimal cardiovascular disease protection [8]. Erythrocyte 18 : 2*n*-6:18 : 3*n*-3 ratios were inversely correlated with erythrocyte 20 : 5*n*-3 contents in vegans (r = -0.541, P = 0.008, n =

23), but not 22 : 5*n*-3 or 22 : 6*n*-3 contents; with no significant correlations in the erythrocyte lipids of omnivores. In plasma, the ratio of 18 : 2*n*-6:18 : 3*n*-3 was inversely correlated with plasma 22 : 5*n*-3 (r = -0. 576, P = 0.004, n = 23) and 22 : 6*n*-3 (r = -0.498, P = 0.016, n = 23) in vegans and plasma 22 : 5*n*-3 only in omnivores (r = -0.474, P = 0.019, n = 24).

Table 3 shows *n-3* and *n-6* PUFA-derived lipid mediators evaluated in the fasting plasma of the two study groups. A complete diagrammatic list of all lipid mediators included in the analysis protocol, including those that were and were not detectable in the plasma of this study population is given in **Figures 2A and 2B**. In general, the lipid mediators derived from *n-6* PUFAs (mainly 20 : 3*n*-6 and 18 : 2*n*-6), and plant-derived *n-3* PUFA (18 : 3*n*-3) were higher in vegans compared to omnivores, and the mediators derived from 20 : 5*n*-3 and 22 : 6*n*-3 were lower in vegans compared to omnivores, showing a clear difference in the lipidomic profile between the groups. SPMs (resolvins, protectins and maresins) were not detectable in the fasting plasma samples. Notably, in vegans there were markedly lower fasting plasma concentrations of 18-HEPE, an 20 : 5*n*-3-derived precursor marker for resolvin E1, and undetectable concentrations of 17-HDHA, a 22 : 6*n*-3-derived precursor marker for resolvin D1, resolvin D2, and PDX, an isomer of protectin D1. 14-HDHA, another mediator arising from 22 : 6*n*-3 and a precursor marker for the macrophage-derived maresin 1, was also much lower in vegan fasting plasma compared to omnivores (Table 3). In summary, these data show that vegans have increased blood concentrations of oxygenated metabolites of 18 : 2*n*-6 and 18 : 3*n*-3 compared to omnivores, and very low or undetectable concentrations of LC *n*-3 PUFA-derived oxygenated metabolites.

Heart rate and heart rate variability

Twenty-four hour

The average duration of the 24 h interbeat interval recording was 21:02 h (95% CI 20:11, 21:52); for day-time analysis it was 13:08 h (95% CI 12:38, 13:38) and for sleep-time analysis it was 5:56 h (95% CI 5:25, 6:27). Only recordings with a minimum of 18 h were included in the 24 h physical activity, IBI, HR and SDNN analysis.

Vegans had higher 24 h HRV than omnivores as shown in **Table 4**: SDNN, SDANN, and VLF were higher in vegans. Differences in these parameters indicate greater variability in longer-phase cycles in the vegan group during the 24 h period, whereas beat-to-beat

(parasympathetically driven) variability (RMSSD, PNN50 and HF) and IBI/HR were not different between groups over the 24 h period. This is substantiated by much greater sleep-time minus day-time differences in mean IBI/HR, indicating that vegans experience a greater drop in heart rate from day to night compared to omnivores, due to having faster day-time heart rates.

Day-time

Day-time analysis was carried out on the first 8 h segment of data following fitting of the monitor on the morning of the study visit, excluding noisy sections where signal was poor, in order to standardise the length of recording. There was no difference in physical activity levels as assessed by accelerometry between vegans and omnivores. IBI was significantly shorter in vegans (reciprocal to heart rate, for which group differences fell just short of statistical significance) during the day compared to omnivores (Table 4). In contrast to the 24 h measurement period, HRV was reduced in vegans during the day compared to omnivores: this was observed in parameters of overall variability (SDNN, LF, although not in triangular index) and the beat-to-beat parameters of variability (RMSSD, PNN50, HF). To summarise, during day-time waking hours, vegans had shorter IBI/faster heart rates and in accordance with this, they had reduced beat-to-beat HRV, compared to omnivores, even after adjusting for physical activity and other covariates.

Nocturnal sleep-time

Sleep-time analysis was carried out on the first 2 h of sleep data, excluding periods of awakening as determined by increases in accelerometry counts per min, in order to standardise the length of recording. Longer segments were not available for all participants and therefore were not included in the analysis. There were no significant differences for any of the parameters between groups.

Nocturnal sleep-time minus day-time differences in heart rate/interbeat intervals and beat-to-beat heart rate variability

Circadian changes are a key determinant of variability in heart rate over 24 h, measured as 24 h SDNN. Differences in mean nocturnal sleep-time and day-time IBIs are a significant factor in the size of the SDNN value. As described above, the sleep-time minus day-time differences in HR/IBI were statistically significant, with the mean decrease in HR/increase in

IBI from day-time to sleep-time being distinctly larger in vegans compared to omnivores (Table 4). The lack of difference in sleep-time heart rate between groups, together with observations of shorter IBI (and non-statistically significant faster HR) during the day in vegans, suggests that the larger night-day difference in vegans is a result of greater circadian fluctuations in sympathetic-parasympathetic balance. Sleep-time minus day-time HRV also reflects the degree of circadian modulation of autonomic regulation of HR in both vegans and omnivores. The larger increases in beat-to-beat HRV parameters (RMSSD, HF, PNN50) during nocturnal sleep may indicate a greater suppression of parasympathetic regulation during day-time waking hours in vegans when considered alongside the shorter mean day-time IBI in this group compared to omnivores.

DISCUSSION

Low HRV is associated with mortality after a myocardial infarction [21; 40; 41] and risk of cardiac events in the general population [23]. Associations between increased *n-3* PUFA consumption and higher HRV[42; 43; 44; 45], and lower heart rates[16], suggests that populations with very low *n*-3 PUFA tissue status might be at greater risk of arrhythmic events or sudden cardiac death. Vegetarians/vegans in the Adventist Health Study [46], EPIC-Oxford cohort[47], and 5 combined cohorts[48] have been reported to have lower risk of coronary heart disease (CHD) than non-vegetarians. However, a recent study of 2 combined population cohorts (EPIC-Oxford and the earlier Oxford Vegetarian Study cohort) reported similar rates of all-cause mortality and no clear differences between vegans and comparable regular meat-eaters, fish-eaters and vegetarians in mortality from CHD up to the age of 90 years [49], despite the fact that vegan populations have lower CHD risk factors such as blood pressure[14; 15], plasma lipids [12; 13] and lower body mass index [10; 11] compared to populations that eat foods of animal origin. Although the latter findings do not preclude a lower risk of premature CHD in vegans, the notion that cardiovascular health of elderly vegans might be further optimised by increased intakes of dietary long chain *n*-3 PUFA remains a possibility.

We hypothesised that a population with low tissue LC *n*-3 PUFA status would have higher heart rates and lower HRV, and vegans were chosen as a clearly defined group that could be considered as a model to test this hypothesis. As expected, we observed marked differences between vegans and omnivores in their tissue *n*-3 PUFA status, as represented by erythrocyte

lipid fatty acid composition (an indicator of longer-term PUFA intake due to the 4-month lifespan of an average red blood cell [50]). These findings were supported by differences in the plasma fatty acid composition and self-reported dietary LC *n-3* PUFA intakes. The average erythrocyte omega-3 index in the omnivore group was lower than indices reported previously for a meat- and fish-eating UK population [51; 52; 53], but differences between the groups studied here were clear-cut. Inverse relationships were observed between erythrocyte 18 : 2*n*-6 : 18 : 3*n*-3 ratios and erythrocyte 20 : 5*n*-3 in the vegan group. This supports existing evidence that higher dietary intakes of 18 : 2*n*-6, an *n*-6 PUFA which is abundant in omnivore diets but even more so in vegan/vegetarian diets[5], may inhibit conversion of 18 : 3*n*-3 to longer chain *n*-3 PUFA[54].

The observed group differences in heart rate and HRV were more complex than hypothesised, mainly due to divergence in night/day differences. Differences in all primary outcome variables - HR/IBI, SDNN (overall HRV), and RMSSD (beat-to-beat HRV) - were observed between groups but the nature of the difference depended on whether analysis was carried out over the full 24 h or only during day-time waking hours. In line with the hypothesis, mean day-time HR was higher/IBI shorter and overall (SDNN) and beat-to-beat HRV (RMSSD, PNN50%, HF) was lower in vegans, even following adjustment for physical activity during the same 8 h period. These observations might indicate that low omega-3 status could lead to either a predominance of sympathetic regulation, a greater withdrawal of parasympathetic activity, or possibly, due to depletion of longer chain *n*-3 PUFA in cardiomyocyte membranes, there is a greater stimulation of pacemaker activity despite a normal level of sympathetic neural transmission during waking hours. However, it is also possible that the differences in HRV observed in vegans and omnivores are unrelated to LC n-3 PUFA tissue status; this would require investigation with a dietary intervention trial. A recent review on omega-3 fatty acids and effects on heart rate and HRV has argued that, according to evidence from animal models, it is more likely that 22 : 6*n*-3 is acting to reduce heart rate via modulation of pacemaker activity rather than changes in cardiac autonomic neural regulation[18], although the role of the 22 : 6*n*-3-derived SPM, neuroprotectin D1 (PD1), in protecting the nervous system from inflammation-related injury shows that 22 : 6*n*-3-dependent physiological mechanisms exist in synapses and neural circuits in order to sustain neuronal function[55; 56]. The stable precursor to PD1 and resolvin D1 (RvD1), 17-HDHA , was not detected in the fasting plasma of any vegan subjects, whereas 9 out of 24

omnivores had detectable concentrations. There were also marked differences in concentrations of long chain n-3 PUFA-derived precursor markers to resolvin E1 (RvE1, from 18-HEPE) and maresin-1 (MaR1, from 14-HDHA). Venous blood plasma concentrations of lipid mediators in whole fasting plasma are likely to be an insensitive marker of capacity for autacoid release and activity in specific sites of inflamed tissue. Nevertheless, higher circulating plasma concentrations of SPM precursor markers may indicate ease of bioavailability for conversion to SPMs at times of need, which presents clear functional implications for populations with low tissue 20 : 5*n*-3 and 22 : 6*n*-3 stores.

Although the vegan group were not deficient in other nutrients that are related to HRV, such as vitamin D [57] and vitamin B12[58], the nature of the study design means that we cannot exclude the influence of other dietary or lifestyle factors associated with the vegan lifestyle. The vegans reported almost half the intake of saturated fatty acids (% energy) as the omnivores, in agreement with results reported in larger vegan populations [59], and correspondingly lower amounts of 16 : 0 as a proportion of total plasma fatty acids and lower serum concentrations of LDL-C. However, these differences are less likely to exert a major influence on cardiac electrophysiology. Animal studies have shown that PUFA-feeding decreased vulnerability to arrhythmia compared to high SFA-feeding without any reduction in the proportion of membrane SFA, and high-MUFA feeding did not reduce arrhythmia compared to high-SFA diets [60]. This suggests that SFA membrane composition is not a major determinant of vulnerability to arrhythmias and addition of LC n-3 PUFA (replacing mainly 18:1 and n-6 PUFAs) might be the most important determinant. In fact our small cross-sectional study showed that erythrocyte SFA proportions were not different between groups and that vegans had lower day-time HRV, and therefore potentially a greater risk of arrhythmia if there was also coronary atherosclerosis present, despite lower saturated fat intake.

There may be other explanations for higher heart rate and reduced HRV in vegans that are not related to *n*-3 PUFA status and were not measured as part of this study, for example, susceptibility to psychological stress (although reduced self-reported stress and anxiety has been observed in 109 vegans compared to 228 omnivores[61]), and job-related activities, and possibly frequency/duration of using a bicycle (which would not have been detected by accelerometry). The effects of physical activity on HRV depend on the type and intensity of activity involved, but higher parasympathetically-regulated HRV parameters are associated

425 with greater levels of habitual physical activity [62]. Since parasympathetically-regulated HRV
426 parameters were lower in vegans during the day-time, then it suggests that either habitual
427 physical activity levels were lower in vegans or some other factor associated with vegan diet
428 and lifestyle, such as the depletion in tissue 20 : 5*n*-3 + 22 : 6*n*-3 content, counteracted the
429 effect of habitual physical activity levels.

430 No differences were observed between groups during a standardised 2 h sleep period.
431 Previous research from our group showed increased longer-phase HRV (SDANN and VLF)
432 in a middle-aged population at moderate risk of CVD during nocturnal sleep following 12
433 months fish oil supplementation at doses of 0.45-1.8 g/d LC *n*-3 PUFA compared to a refined
434 olive oil placebo[31]. Consistent with this, fish consumption was positively related to VLF in a
435 large cohort of older adults[25]. Low VLF is associated with increased risk of mortality post-
436 myocardial infarction, particularly arrhythmic death[40]. Since SDANN and VLF represent
437 slowly changing periodic variability in heart rate in response to thermoregulatory and
438 hormonal shifts that may particularly occur during sleep, then it is likely that the 2 h
439 standardised period in the current study was too short to detect longer-phase differences in
440 HRV between vegans and omnivores during sleep.

441 Contrasting observations were made for longer-phase components of 24 h HRV, which
442 represent changes in heart rates over sustained periods in response to periodic fluctuations in
443 neurohormonal and circadian physiology rather than beat-to-beat variability. These
444 components of HRV (SDNN, SDANN, and VLF) were higher, and Poincaré ratio was lower,
445 over 24 h in vegans compared to omnivores; this may represent more pronounced
446 neurohormonal rhythms in vegans, or they may just reflect the higher heart rate and reduced
447 HRV experienced by vegans during waking hours relative to sleep-time due to reasons
448 discussed above.

449 Sub-clinical markers of inflammation have been linked to risk of cardiovascular events,
450 vascular inflammation being the key, self-amplifying component of atherogenesis [63; 64; 65].
451 Resolution of acute inflammatory responses is a critical, programmed factor in tissue repair
452 and prevention of further pathological changes to tissues. SPMs derived from long chain *n*-3
453 PUFA take over from the initiating lipid mediators, prostaglandins and leukotrienes, during
454 the neutrophil-monocyte sequence, and play a functional role in ending acute inflammatory
455 events by inhibition of neutrophil influx to the site of trauma, counter-regulating pro-

inflammatory cytokines, and stimulating resolving macrophages to clear the products of the inflammatory response, thereby allowing the injured area to heal[66]. In theory, low tissue availability of 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3 could compromise resolution of acute inflammatory events increasing risk of chronic inflammation, although this is purely speculative at present. Increased circulating concentrations of RvE1 and precursor markers of resolvins (18-HEPE, 17-HDHA), and maresins (14-HDHA) have been demonstrated following *n*-3 PUFA supplementation[67], the same precursor markers that were found to be different in our comparison of vegans and omnivores. Our data show that a population with no dietary intake of marine *n-3* PUFA have much lower or zero fasting plasma concentrations of these SPM precursor markers. It is not known whether individuals with low omega-3 status have increased rates of 20 : 5*n*-3 /22 : 6*n*-3-derived mediator turnover as an adaptive mechanism to avoid compromising SPM availability. If this were the case, then it would be expected that having low pools of SPM precursors would have no functional consequences in vegans. Future research in this area should address whether populations with low-omega-3 status are more at risk of having a pro-inflammatory profile.

Vegans had greater concentrations of 18 : 3*n-3* and 18 : 2*n-6* derived lipid mediators that have a variety of deleterious and cytoprotective effects [68; 69]. In the case of 18 : 2*n-6*, this is likely to be due to higher dietary intakes, as supported by FFQ estimates, proportions of total plasma fatty acids, and incorporation into erythrocyte membrane lipids [68; 70]. Although plasma 18 : 3*n*-3 proportions of total fatty acids were higher in vegans, reported dietary intakes were not different; however, FFQ estimates of intakes are likely to underestimate true intakes due to incomplete food composition data. Vegans also had lower concentrations of markers of AA-derived prostanoid production (6-keto $PGF_{1\alpha}$ – a marker of PGI_2 synthesis, and 13,14-dihydro $PGF_{2\alpha}$,/13,14-dihydro-15-keto $PGF_{2\alpha}$ - markers of $PGF_{2\alpha}$ production). There were no differences between groups for a range of AA-derived LOX-catalysed mediators (HETEs), suggesting that the lipid mediator profile of vegans may not necessarily be entirely pro-inflammatory relative to omnivores. Few of these lipid mediators have been fully characterised regarding their functional effects, and evidence in animal and cell models to date suggests that 18 : 2*n*-6- and 20 : 4*n*-6-derived lipid mediators comprise a complex array of diverse bioactive molecules that induce a range of physiological effects in various tissues [71; 72; 73].

Previous work has also demonstrated that circulating pro-inflammatory cytokines may be reduced by fish oil supplementation, as reviewed by Calder [74; 75]. We included a measure of low grade inflammation, IL-6, in our comparison between vegans and omnivores, but found no differences between groups. However, this does not necessarily indicate that there are no differences between groups in their capacity to inhibit or resolve acute inflammatory events since circulating cytokine concentrations have limited utility as biomarkers of inflammation that may be occurring in localised areas of tissue. Previous studies have shown that serum IL-6 concentrations were inversely correlated with HRV in men with renal disease [76], metabolic syndrome [77] and young healthy subjects [78], although not not all studies agree [79]. Downregulation of inflammatory cytokine gene expression plus increased production of pro-resolving lipid mediators are two potential mechanisms whereby cardiac function might possibly be preserved by increased 20 : 5*n*-3 and 22 : 6*n*-3 intakes, by reducing inflammatory tissue damage in the brain and autonomic nerves, and also in the heart tissue itself.

Limitations of the present study.

The cross-sectional design limits our findings to being exploratory in nature and the associations between low omega-3 status and reduced HRV require confirmation by a randomised controlled trial of 20 : 5*n*-3 + 22 : 6*n*-3 supplementation in a population with an omega-3 index of <3%. The sample population size is small and although statistical power calculations were conducted for the primary HRV outcomes, the study may be underpowered to detect group differences in other more variable outcomes such as beat-to-beat heart rate. Multiple statistical testing was carried out to explore group differences in short- and long-term, and time and frequency domain HRV, increasing the risk of generating false-positive results. There is no agreed upon method for correcting statistical analyses that involve the full set of HRV measures, but the dataset represents groupings of related outcomes rather than a large collection of disparate variables. The data presented here is consistent when comparing variables that represent similar physiological phenomenon. For example, there are two time domain (RMSSD, pNN50) and one frequency domain (HF power) parameters of beat-to-beat variability. These are all vagally regulated and all show consistently that day-time beat-to-beat HRV is lower in vegans compared to omnivores. Therefore, although Type I errors cannot be ruled out with complete certainty, it is reassuring that statistically significant differences between groups are supported by analogous parameters. The stated aim was to match groups for age, sex and BMI, but matching for sex was not wholly achieved. Any

influence of this imbalance in sex distribution on HRV results was minimised by adjusting for age, sex and BMI, in addition to activity levels for 24 h and day-time HRV, in the statistical model. Technical problems in obtaining good quality sleep-time HRV data limited the standardised duration of nocturnal HRV to 2 h which may have led to effects on longer phase HRV parameters being missed. However, the fact that heart rate variability was lower and mean IBI was shorter in vegans only during the day, and not over the whole 24 h period, suggests that there may be a diet-mental stress interaction during waking hours that resulted in a greater degree of sympathetic nervous system activity relative to parasympathetic activity. Future studies could investigate this further by measuring HRV responses under controlled mental stress conditions in populations with very low omega-3 indices compared to populations with optimum omega-3 indices.

Summary

The differences observed in parameters of cardiac electrophysiology and circulating lipid mediator concentrations between vegans and omnivores may contribute to the sum effect of diet and lifestyle on cardiovascular disease risk. The lower availability of long chain n-3 PUFA-derived lipid mediators in vegans may influence anti-inflammatory capacity, although other differences in linoleic and α-linolenic acid-derived mediators feed into an array of disparate inflammatory pathways and the sum effect is difficult to predict. Crucially, this study presents novel information on associations between free-living, unsupplemented dietary PUFA intakes with lipid mediator profiles in humans.

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547

548 CONFLICT OF INTEREST

549 None

550

551 AUTHORSHIP

552 WLH, TABS and AMP conceived the research question and devised the study. AMP
553 conducted the study and analysed the data, with the assistance of HA-K. AN, ACK and RG
554 provided lipidomic analytical expertise. All authors contributed to writing and editing the
555 manuscript.

FIGURE TITLES AND LEGENDS

FIGURE 1. Consort diagram.

(no legend)

FIGURE 2A. Schematic outline of oxygenated species derivatives of *n*-6 polyunsaturated fatty acids, linoleic acid (LA; 18 : 2*n*-6), dihomo-γ-linolenic acid (DGLA; 20 : 3*n*-6) and arachidonic acid (AA; 20 : 4*n*-6), analysed in blood plasma of vegans and omnivores. B. Schematic outline of oxygenated species derivatives of *n*-3 polyunsaturated fatty acids, α-linolenic acid (ALA; 18 : 3*n*-3), eicosapentaenoic acid (EPA; 20 : 5*n*-3) and docosahexaenoic acid (DHA; 22 : 6*n*-3), analysed in blood plasma of vegans and omnivores.

Legend for FIGURE 2A:

20 : 3*n*-6 (DGLA)-, 18 : 2*n*-6 (LA)- and 20 : 4*n*-6 (AA)-derived lipid mediators assayed in study participants' plasma. Red = higher in vegans, blue = higher in omnivores, purple = no difference, white = not detected or below the limit of detection. HODE, hydroxyoctadecadienoic acid; OxoODE, oxooctadecadienoic acid; EKODE, epoxyketooctadecenoic acid; EpOME, epoxyoctadecenoic acid; DiHOME, dihydroxyoctadecenoic acid; PG, prostaglandin ; HETrE, hydroxyeicosatrienoic acid; TX, thromboxane; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatetraenoic acid; DHET, dihydroeicosatetraenoic acid; DiHETE,dihydroxyeicosatetraenoic acid ; HX, hepoxilin ; LT, leukotriene.

Legend for FIGURE 2B:

18 : 3*n*-3 (ALA)-, 20 : 5*n*-3 (EPA)- and 22 : 6*n*-3 (DHA)-derived lipid mediators assayed in study participants' plasma. Red = higher in vegans, blue = higher in omnivores, purple = no difference, white = not detected or below the limit of detection. HOTrE, hydroxyoctadecatrienoic acid; TX, thromboxane; PG, prostaglandin; HEPE, hydroxyeicosapentaenoic acid; Rv, resolvin; HDHA, hydroxydocosahexaenoic acid; PD,

582 protectin D; MaR, maresin; EpDPE, epoxydocosapentaenoic acid;
583 DiHDPA,dihydroxydocosapentaenoic acid.

TABLE 1. Characteristics and background dietary intakes of vegan and omnivore participants

	Omnivore (n=24)		Vegan (n=23)		*P* value[‡]
	Number	**%**	**Number**	**%**	
Gender					
Male	12	50	8	35	0.292[§]
Female	12	50	15	65	
Ethnicity					
White	21	88	21	91	0.289[§]
Black African/Caribbean	0	0	1	5	
South Asian	2	8	0	0	
Other	1	4	1	5	
Vitamin B12 supplement use	8	33	14	61	0.059[§]
	Mean/geometric mean[*]/median[†]	SD/95% CI/IQR	Mean/geometric mean[*]/median[†]	SD/95% CI/IQR	
Age (years)	54	9.1	49	7.9	0.081

	Omnivore (n=24)		Vegan (n=23)		*P* value[‡]
BMI (kg/m^2)	23.3	2.8	23.5	4.4	0.896
Waist circumference (cm)					
Male	91.7	6.5	97.2	5.9	0.165
Female	88.8	12.5	86.0	14.8	0.617
% body fat					
Male	19.3	7.9	21.1	5.9	0.572
Female	30.6	7.7	29.4	9.9	0.751
Systolic BP (mmHg)	118	9.8	111	9.9	0.032
Diastolic BP (mmHg)	75	9.3	74	8.2	0.703
Heart Rate (bpm)	63	10.8	70	9.0	0.017
Plasma glucose (mmol/L)	5.2	0.4	5.1	0.4	0.616
Serum triacylglycerol (mmol/L) [*]	0.77	0.66, 0.92	0.76	0.64, 0.90	0.849
Serum total cholesterol (mmol/L)	4.9	0.86	4.1	0.66	0.001
Serum LDL-C	2.81	2.49, 3.17	2.16	1.94,	0.002

	Omnivore (n=24)		Vegan (n=23)		*P* value[‡]
(mmol/L) [*]				2.41	
Serum HDL-C (mmol/L)					
Male	1.52	0.35	1.29	0.23	0.118
Female	1.71	0.24	1.66	0.42	0.725
Energy intake (MJ)	8.17	2.00	7.67	2.77	0.477
Protein intake (%E)	16.6	2.2	13.3	2.4	< 0.001
Carbohydrate intake (%E)	49.2	7.3	56.5	11.6	0.013
Total fat intake (%E)	33.8	5.9	30.9	9.5	0.216
SFA intake (%E)	11.8	2.6	6.3	1.7	< 0.001
MUFA intake (%E) [†]	13.5	12.1,15.0	11.6	9.3,14.4	0.136 [‖]
PUFA intake (%E)	6.0	1.2	9.6	3.1	< 0.001 [‖]
18 : 2*n*-6 intake (g) [†]	7.6	5.9, 10.2	10.5	7.3, 18.5	0.025 [‖]
18 : 3*n*-3 intake (g) [†]	0.7	0.5, 1.0	0.8	0.5, 1.2	0.425
20 : 5*n*-3 intake (g) [†]	0.14	0.09, 0.24	0.00	0.00, 0.00	< 0.001 [‖]

	Omnivore (n=24)		Vegan (n=23)		*P* value[‡]
22 : 6*n*-3 intake (g) [†]	0.45	0.30, 0.81	0.01	0.01, 0.01	< 0.001 [‖]
Serum vitamin B_{12} (ng/L)	442	216	358	117	0.108
Haemoglobin (g/L)					
Male	14.4	0.6	14.4	0.8	0.805
Female	13.2	1.0	13.5	1.0	0.460
Serum 25-hydroxy vitamin D (nmol/L)	54.3 [**]	20.9	55.6	26.5	0.854
Interleukin-6 (ng/L) [†]	0.93 [**]	0.25, 1.54	1.07	0.23, 3.05	0.701[‖]

BMI. body mass index; BP, blood pressure; bpm, beats per minute; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; 18 : 2*n*-6 (LA), linoleic acid; 18 : 3*n*-3 (ALA), alpha-linolenic acid; 20 : 5*n*-3 (EPA), eicosapentaenoic acid; 22 : 6*n*-3 (DHA), docosahexaenoic acid.

Results are expressed as number of subjects (percentage) or mean with standard deviation, except where [*] denotes geometric mean with 95% confidence intervals, and [†] denotes median with upper and lower quartiles.

[‡] shows use of independent samples *t*-test, except [§] Chi Square Test and [‖] Mann-Whitney U test. [**] n=23 due to sample loss.

TABLE 2. Plasma and erythrocyte fatty acid composition in vegan and omnivore participants (*n*=47).

	Omnivore (*n*=24)		Vegan (*n*=23)		Difference between groups		
Plasma and erythrocyte fatty acids (weight %)	Mean/geometric mean*/median†	95% CI/IQR	Mean/geometric mean*/median†	95% CI/IQR	Mean difference‡	95% CI	*P* value‖
Plasma							
16 : 0	20.8	20.4, 21.3	19.3	18.6, 20.0	-1.49	-2.31, -0.67	0.001
16 : 1*n*-7	1.79	1.48, 2.10	1.11	0.91, 1.32	-0.67	-1.04, -0.31	0.001
18 : 0	7.62	7.30, 7.94	7.60	7.15, 8.04	-0.02	-0.55, 0.50	0.931
18 : 1*n*-9	18.5	17.6, 19.3	18.9	17.9, 19.8	0.37	-0.89, 1.62	0.559
18 : 2*n*-6	27.1	26.0, 28.2	33.1	31.9, 34.4	6.06	4.43, 7.68	< 0.001
18 : 3*n*-3 *	0.53	0.48, 0.59	0.71	0.59, 0.85	1.34§	1.09, 1.64	0.006

	Omnivore (*n*=24)		Vegan (*n*=23)		Difference between groups		
Plasma and erythrocyte fatty acids (weight %)	Mean/geometric mean*/median†	95% CI/IQR	Mean/geometric mean*/median†	95% CI/IQR	Mean difference‡	95% CI	*P* value‖
20 : 3*n*-6	1.42	1.28, 1.55	1.42	1.28, 1.57	0.01	-0.18, 0.20	0.952
20 : 4*n*-6	6.68	6.12, 7.25	6.55	5.94, 7.16	-0.13	-0.94, 0.68	0.745
20 : 5*n*-3 *	1.03	0.79, 1.34	0.47	0.40, 0.55	0.46§	0.34, 0.62	< 0.001
22 : 4*n*-6 *	0.20	0.19, 0.21	0.23	0.21, 0.25	1.14§	1.01, 1.28	0.036
22 : 5*n*-6	0.26	0.21, 0.30	0.20	0.15, 0.26	-0.05	-0.12, 0.02	0.146
22 : 5*n*-3	0.59	0.53, 0.64	0.51	0.44, 0.59	-0.07	-0.16, 0.02	0.113
22 : 6*n*-3 *	2.23	1.94, 2.57	0.91	0.80, 1.05	0.41§	0.34, 0.49	< 0.001
Erythrocyte							

	Omnivore (*n*=24)		Vegan (*n*=23)		Difference between groups		
Plasma and erythrocyte fatty acids (weight %)	Mean/geometric mean*/median†	95% CI/IQR	Mean/geometric mean*/median†	95% CI/IQR	Mean difference‡	95% CI	*P* value‖
16 : 0	16.8	15.4, 18.2	17.6	16.7, 18.6	0.81	-0.87, 2.48	0.337
16 : 1*n*-7 †	0.41	0.30, 1.57	0.31	0.21, 0.50	-	-	0.016¶
18 : 0 *	15.6	14.9, 16.3	16.3	15.6, 17.1	1.05§	0.99, 1.14	0.135
18 : 1*n*-9	15.7	15.1, 16.3	15.4	14.7, 16.2	-0.23	-1.15, 0.68	0.609
18 : 2*n*-6	11.7	11.0, 12.3	13.3	12.5, 14.1	1.64	0.64, 2.64	0.002
18 : 3*n*-3 *	0.34	0.26, 0.45	0.32	0.27, 0.38	0.92§	0.67, 1.27	0.610
20 : 3*n*-6 *	1.78	1.64, 1.94	2.02	1.84, 2.22	1.13§	1.01, 1.28	0.042
20 : 4*n*-6	15.9	14.9, 16.9	15.6	14.4, 16.9	-0.27	-1.82, 1.27	0.725

	Omnivore (*n*=24)		Vegan (*n*=23)		Difference between groups		
Plasma and erythrocyte fatty acids (weight %)	Mean/geometric mean*/median†	95% CI/IQR	Mean/geometric mean*/median†	95% CI/IQR	Mean difference‡	95% CI	*P* value‖
20 : 5*n*-3	1.26	1.07, 1.45	0.67	0.52, 0.81	-0.59	-0.83, -0.36	< 0.001
22 : 4*n*-6	2.75	2.47, 3.03	3.83	3.50, 4.16	1.08	0.66, 1.50	< 0.001
22 : 5*n*-6	0.38	0.27, 0.49	0.52	0.40, 0.64	0.14	-0.02, 0.30	0.078
22 : 5*n*-3	2.62	2.36, 2.88	2.15	1.94, 2.36	-0.47	-0.80, -0.15	0.005
22 : 6*n*-3 *	4.19	3.63, 4.83	2.07	1.85, 2.32	0.49§	0.41, 0.59	< 0.001
Omega 3 index *	5.42	4.73, 6.20	2.71	2.40, 3.05	0.50§	0.42, 0.60	< 0.001

16 : 0, palmitic acid; 16 : 1*n*-7, palmitoleic acid; 18 : 0, stearic acid; 18 : 1*n*-9, oleic acid; 18 : 2*n*-6, linoleic acid; 18 : 3*n*-3, α-linolenic acid; 20 : 3*n*-6, dihomo-γ-linolenic acid; 20 : 4*n*-6, arachidonic acid; 20 : 5*n*-3, eicosapentaenoic acid; 22 : 5*n*-3, docosapentaenoic acid *n*-3; 22 : 6*n*-3, docosahexaenoic acid. Results are expressed as mean (95% CI), except where * denotes geometric means (95% CI) and † denotes median (lower and upper quartiles). ‡Mean Δ vegan - omnivore (95% CI), except where § denotes exponents of mean differences in Ln values (the ratio of the geometric mean in vegans to that in omnivores, with 95% CI of the geometric mean ratios). ‖ P value obtained using independent samples *t*-test,

except where ¶ denotes use of Mann-Whitney U test where data remained not normally distributed following LN transformation. Total plasma fatty acid concentrations were (geometric means with 95% CI): omnivores (1869 mg/L, 1660, 2104; n=24), vegans (1998 mg/L, 1755, 2274; n=23); there were no significant differences between groups.

TABLE 3. Plasma concentrations of *n-6* and *n-3* PUFA-derived lipid mediators in vegan and omnivore participants (n=47).

Compound (ng/L)	Omnivore (*n*=24)		Vegan (*n*=23)		*P* value‖
	Mean/geometric mean*/median†	95% CI/minimum and maximum	Mean/geometric mean*/median†	95% CI/minimum and maximum	
***n-6* PUFA-derived**					
20 : 3*n*-6-derived					
15-HETrE	51.82	36.94, 66.70	53.87	39.78, 67.96	0.533
13,14-dihydro-15-keto PGE_1 *	17.45	9.52, 31.99	13.93	7.82, 24.78	0.519
13,14-dihydro PGE_1 †	0.000	0.000, 4.332	0.000	0.000, 76.928	0.001¶
18 : 2*n*-6-derived					
9-HODE *	2433	1982, 2988	5045	4067, 6260	<0.001
9 OxoODE *	477	398, 571	994	771, 1282	<0.001
13-HODE *	3320	2717, 4056	6536	5483, 7791	<0.001
13 OxoODE	335	291, 379	537	467, 606	<0.001

Compound (ng/L)	Omnivore (*n*=24)		Vegan (*n*=23)		*P* value‖
	Mean/geometric mean*/median†	95% CI/minimum and maximum	Mean/geometric mean*/median†	95% CI/minimum and maximum	
12,13-EpOME	389	326, 451	769	622, 917	<0.001
12,13-DiHOME	2820	2159, 3480	5544	4527, 6561	<0.001
9,10-EpOME *	258	220, 303	426	351, 518	<0.001
9,10-DiHOME *	3199	2482, 4125	7400	5910, 9267	<0.001
Trans EKODE *	300	248, 362	560	425, 738	<0.001
20 : 4*n*-6-derived					
6-keto $PGF_{1\alpha}$ †	8.20	0.000, 59.14	0.000	0.000, 4.49	<0.001¶
13,14-dihydro $PGF_{2\alpha}$ *	48.4	34.57, 67.91	20.0	14.82, 26.97	<0.001
13,14-dihydro-15-keto $PGF_{2\alpha}$ †	0.000	0.000, 25.56	0.000	0.000, 12.79	0.016¶
13,14-dihydro-15-keto PGE_2 *	3.67	2.584, 5.217	3.07	2.172, 4.347	0.459
TXB2 *	10.3	7.57, 14.14	10.5	6.08, 18.05	0.968

Compound (ng/L)	Omnivore (*n*=24)		Vegan (*n*=23)		*P* value‖
	Mean/geometric mean*/median†	95% CI/minimum and maximum	Mean/geometric mean*/median†	95% CI/minimum and maximum	
5-HETE *	124	96.4, 160.4	132	103.2, 168	0.738
8-HETE	70.2	56.77, 83.57	74.5	62.33, 86.60	0.626
9-HETE †	12.82	0.00, 70.99	0.000	0.000, 70.99	0.478¶
11-HETE	62.3	49.55, 75.13	59.7	49.68, 69.64	0.736
12-HETE †	146.48	96.76, 1214.43	143.6	82.20, 1625.76	0.580¶
15-HETE	174	145, 203	186	158, 214	0.533
20-HETE *	284	232, 348	290	229, 367	0.896
5,6-DHET *	55.4	47.09, 65.19	52.0	41.29, 65.37	0.635
8,9-DHET *	71.6	62.75, 81.81	82.1	67.40, 99.95	0.238
11,12-DHET	204	173, 235	233	207, 260	0.147
14,15-DHET	260	231, 290	312	272, 353	0.036

Compound (ng/L)	Omnivore (*n*=24)		Vegan (*n*=23)		*P* value‖
	Mean/geometric mean*/median†	95% CI/minimum and maximum	Mean/geometric mean*/median†	95% CI/minimum and maximum	
***n-3* PUFA-derived**					
18 : 3n-3-derived					
9-HOTrE *	139	111, 173	206	170, 250	0.007
13-HOTrE *	150	114, 198	245	201, 299	0.005
20 : 5*n*-3-derived					
5-HEPE†	75.7	0.000, 462.2	21.4	0.000, 107.5	<0.001¶
8-HEPE†	15.2	0.000, 219.0	0.000	0.000, 21.0	<0.001¶
18-HEPE†	74.8	0.000, 624.8	0.000	0.000, 150.2	<0.001¶
22 : 6*n*-3-derived					
4-HDHA†	75.800	38.181, 413.405	45.734	0.000, 102.331	<0.001¶

Compound (ng/L)	Omnivore (*n*=24)		Vegan (*n*=23)		*P* value‖
	Mean/geometric mean*/median†	95% CI/minimum and maximum	Mean/geometric mean*/median†	95% CI/minimum and maximum	
7-HDHA†	0.000	0.000, 202.374	0.000	0.000, 125.813	0.581¶
10-HDHA†	20.529	0.000, 197.107	0.000	0.000, 14.974	<0.001¶
11-HDHA†	0.000	0.000, 191.074	0.000	0.000, 0.000	0.022¶
13-HDHA†	19.255	0.000, 87.861	0.000	0.000, 0.000	<0.001¶
14-HDHA†	41.255	0.000, 299.686	0.000	0.000, 57.819	<0.001¶
17-HDHA†	0.000	0.000, 364.505	0.000	0.000, 0.000	0.001¶
20-HDHA†	107.538	44.088, 748.889	25.446	0.000, 105.464	<0.001¶
19,20- DiHDPA *	1448	1240, 1690	1098	878, 1374	0.040

20 : 3*n*-6, dihomo-γ-linolenic acid; HETrE, hydroxyeicosatrienoic acid; PG, prostaglandin; 18 : 2*n*-6, linoleic acid; HODE, hydroxyoctadecadienoic acid; OxoODE, oxooctadecadienoic acid; EpOME, epoxyoctadecenoic acid; DiHOME, dihydroxyoctadecenoic acid; EKODE, epoxyketooctadecenoic acid; 20 : 4*n*-6, arachidonic acid; TX, thromboxane; HETE, hydroxyeicosatetraenoic acid; DHET, dihydroeicosatetraenoic acid; 18 : 3*n*-3, α-linolenic acid; HOTrE, hydroxyoctadecatrienoic acid; 20 : 5*n*-3, eicosapentaenoic acid; HEPE,

hydroxyeicosapentaenoic acid; 22 : 6*n*-3, docosahexaenoic acid; HDHA, hydroxydocosahexaenoic acid; DiHDPA, dihydroxydocosapentaenoic acid.

Results are expressed as mean (95% CI), except where * denotes geometric means (95% CI) and † denotes median (minimum and maximum values).

‖ P value obtained using independent samples *t*-test, except where ¶ denotes use of Mann-Whitney U test where data remained not normally distributed following LN transformation.

TABLE 4. Physical activity, heart rate and heart rate variability parameters of vegan and omnivore participants over 24 h, day-time and sleep-time, with sleep – day differences (n=47).

	Omnivore (n = 24)	Vegan (n = 22[*])	*P* [§]
	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	
Twenty-four hour measurement			
Activity, IBI and HR			
Physical activity (cpm)[¶††]	236 (196, 284)	285 (233, 348)	0.181
IBI (ms)[††]	845 (798, 892)	811 (760, 861)	0.336
HR (bpm)[††]	75 (71, 79)	78 (74, 83)	0.210
Time-domain HRV parameters			
Ti	38 (33, 42)	41 (36, 46)	0.294
SDNN (ms)[††]	145 (129, 162)	172 (154, 189)	0.039
SDANN (ms)	128 (114, 143)	155 (139, 170)	0.018
RMSSD (ms)[¶]	35 (31, 40)	35 (30, 40)	0.905
PNN50 (%)[¶]	8.9 (6.5, 12.2)	7.0 (5.0, 9.7)	0.299
Frequency-domain HRV parameters			

	Omnivore (n = 24)	Vegan (n = 22[*])	*P* [§]
	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	
LF (ms^2) [¶]	971 (786, 1198)	761 (608, 953)	0.130
HF (ms^2) [¶]	350 (262, 467)	309 (227, 421)	0.567
VLF (ms^2) [¶]	12619 (10351, 15379)	17980 (14547, 22204)	0.022
Non-linear methods			
SD1:SD2 (Poincaré ratio)	0.13 (0.11, 0.14)	0.11 (0.10, 0.12)	0.051
Day-time (8 hours) measurement			
Activity, IBI and HR			
Physical activity (cpm)[¶]	437 (347, 552)	443 (345, 570)	0.935
IBI (ms)	787 (745, 830)	721 (675, 766)	0.039
HR (bpm)	80 (76, 84)	86 (81, 91)	0.062
Time-domain HRV parameters			
Ti	31 (28, 34)	27 (24, 31)	0.135
SDNN (ms)	121 (109, 132)	101 (89, 113)	0.021

	Omnivore (n = 24)	Vegan (n = 22[*])	*P* [§]
	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	
SDANN (ms)	100 (89, 111)	84 (73, 96)	0.056
RMSSD (ms)	34 (30, 38)	25 (20, 30)	0.009
PNN50 (%) [¶]	6.6 (4.6, 9.4)	2.7 (1.8, 3.9)	0.001
Frequency-domain HRV parameters			
LF (ms^2)	908 (785, 1032)	628 (495, 761)	0.004
HF (ms^2) [¶]	260 (198, 342)	135 (100, 181)	0.002
VLF (ms^2) [¶]	8325 (6894, 10052)	6470 (5277, 7929)	0.078
Non-linear methods			
SD1:SD2 (Poincaré ratio)	0.15 (0.13, 0.16)	0.13 (0.11, 0.15)	0.125
Sleep-time (2 hours) measurement			
IBI and HR			
IBI (ms)	965 (903, 1026)	991 (925, 1056)	0.568
HR (bpm)	64 (61, 68)	62 (59, 66)	0.475
Time-domain HRV parameters			

	Omnivore (n = 24)	Vegan (n = 22[*])	*P* [§]
	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	
Ti	19 (16, 22)	18 (16, 21)	0.762
SDNN (ms)	78 (68, 88)	85 (75, 96)	0.324
SDANN (ms) [¶]	42 (36, 51)	44 (37, 54)	0.717
RMSSD (ms) [¶]	38 (32, 45)	44 (37, 53)	0.214
PNN50 (%) [¶]	10.9 (7.0, 16.9)	11.7 (7.3, 18.7)	0.826
Frequency-domain HRV parameters			
LF (ms^2) [¶]	882 (646, 1205)	902 (647, 1256)	0.925
HF (ms^2) [¶]	403 (280, 580)	464 (315, 684)	0.601
VLF (ms^2) [¶]	2881 (2215, 3744)	3519 (2661, 3744)	0.304
Non-linear methods			
SD1:SD2 (Poincaré ratio) [¶]	0.27 (0.23, 0.32)	0.28 (0.24, 0.33)	0.740
Sleep-time – day-time differences			
IBI and HR			
IBI (ms)	183 (139, 227)	270 (223, 317)	0.012

	Omnivore (n = 24)	Vegan (n = 22[*])	*P* [§]
	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	Mean/geometric mean[¶]/median[**] (95% CI/IQR)	
HR (bpm)	-16 (-19, -13)	-24 (-28, -20)	0.003
Time-domain beat-to-beat HRV parameters			
RMSSD (ms)	8 (1, 14)	22 (15, 29)	0.006
PNN50 (%)	6.4 (0.9, 11.8)	14.2 (8.5, 20.1)	0.058
Frequency-domain beat-to-beat HRV parameters			
HF (ms^2) [**]	127 (-12, 417)	234 (84, 988)	0.095[‖]

Cpm, counts per minute; IBI, interbeat interval (also known as RR interval), the time interval between R spikes of the QRS complex; bpm, beats per minute; HRV, heart rate variability; Ti, total number of all NN intervals divided by the height of the histogram of all NN intervals; SDNN, standard deviation of all NN intervals (normal-to-normal intervals, similar to R-R, but on normalized IBI data); ms, milliseconds; SDANN, standard deviation of the averaged NN intervals, calculated from 5 min epochs; RMSSD, the square root of the mean of the sum of squares of differences between adjacent NN intervals; PNN50, percentage of adjacent NN intervals that differed by more than 50%; LF, low frequency power; HF, high frequency power; VLF, very low frequency power; SD1:SD2, the ratio of the SD of beat-to-beat IBI variability (SD1) against the SD of long-term IBI variability (SD2).

Results expressed as estimated marginal means (95% CI), adjusted for gender, age, BMI and 24 h activity for 24 h HRV and sleep-time – day-time HRV, or 8 h activity for 8 h day-time, and adjusted for gender, age and BMI only for sleep-time. Sleep-time – day-time represents HR/IBI and beat-to-beat HRV during a standardized 2 h nocturnal sleep period minus a standardized 8 h day-time period, to indicate the difference between night and day.

[*]Missing data from 1 subject due to unusable day-time HRV recording.

[§] P value obtained using analysis of covariance for normally distributed raw or LN transformed data (adjusted for gender, age, BMI and activity for 24 h, day-time and sleep-time – day-time differences, and adjusted for gender, age and BMI only for sleep-time), except for sleep-time – day-time differences in HF, where [‖] denotes use of an unadjusted non-parametric test, the Mann-Whitney U test, where data remained not normally distributed following LN transformation. [¶] denotes geometric means (95% CI) and [**] denotes medians (lower and upper quartiles).

[††] Only recordings >18 h included for 24 h physical activity, SDNN, IBI and HR data analysis, *n* 21 for omnivores and 19 for vegans.

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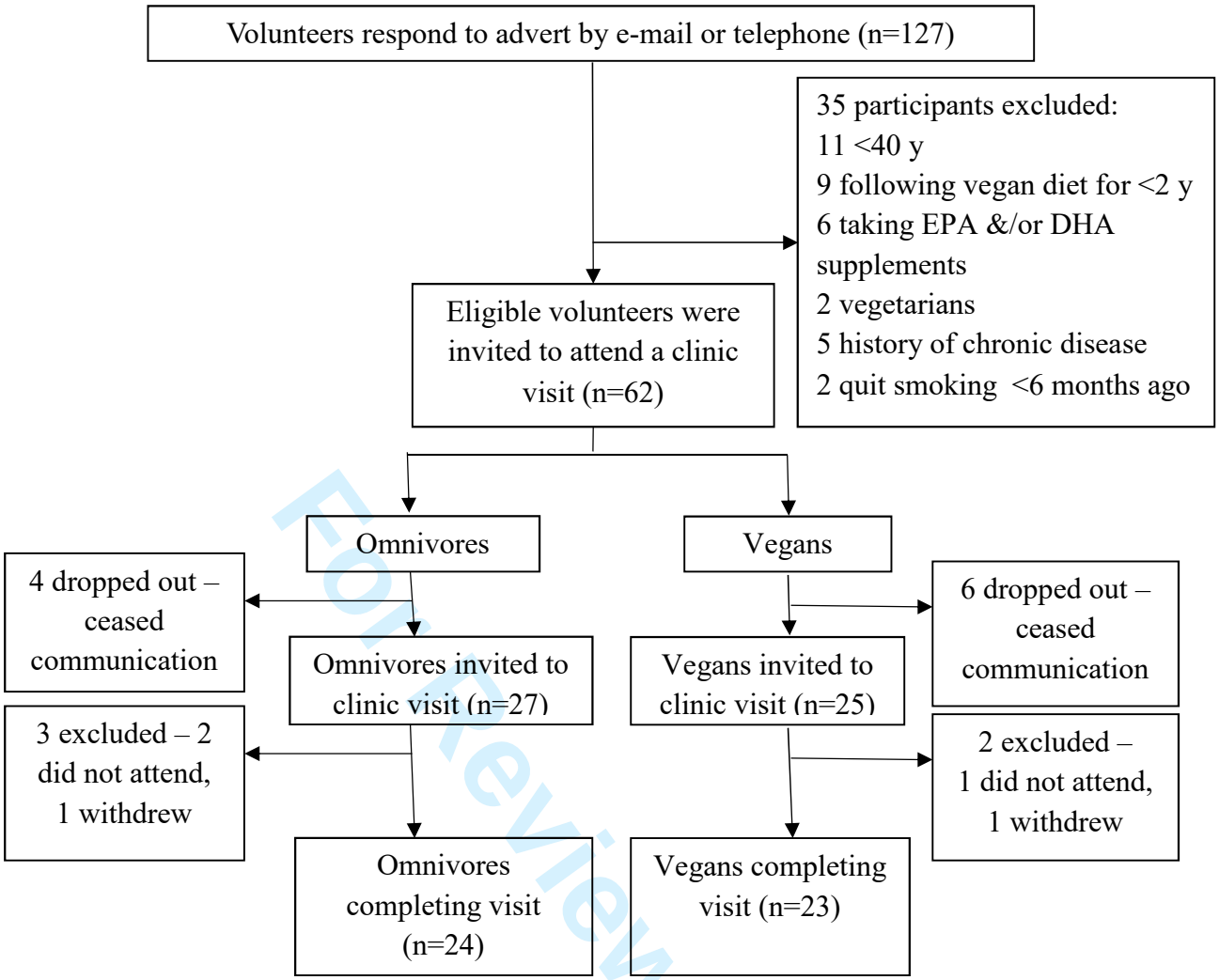
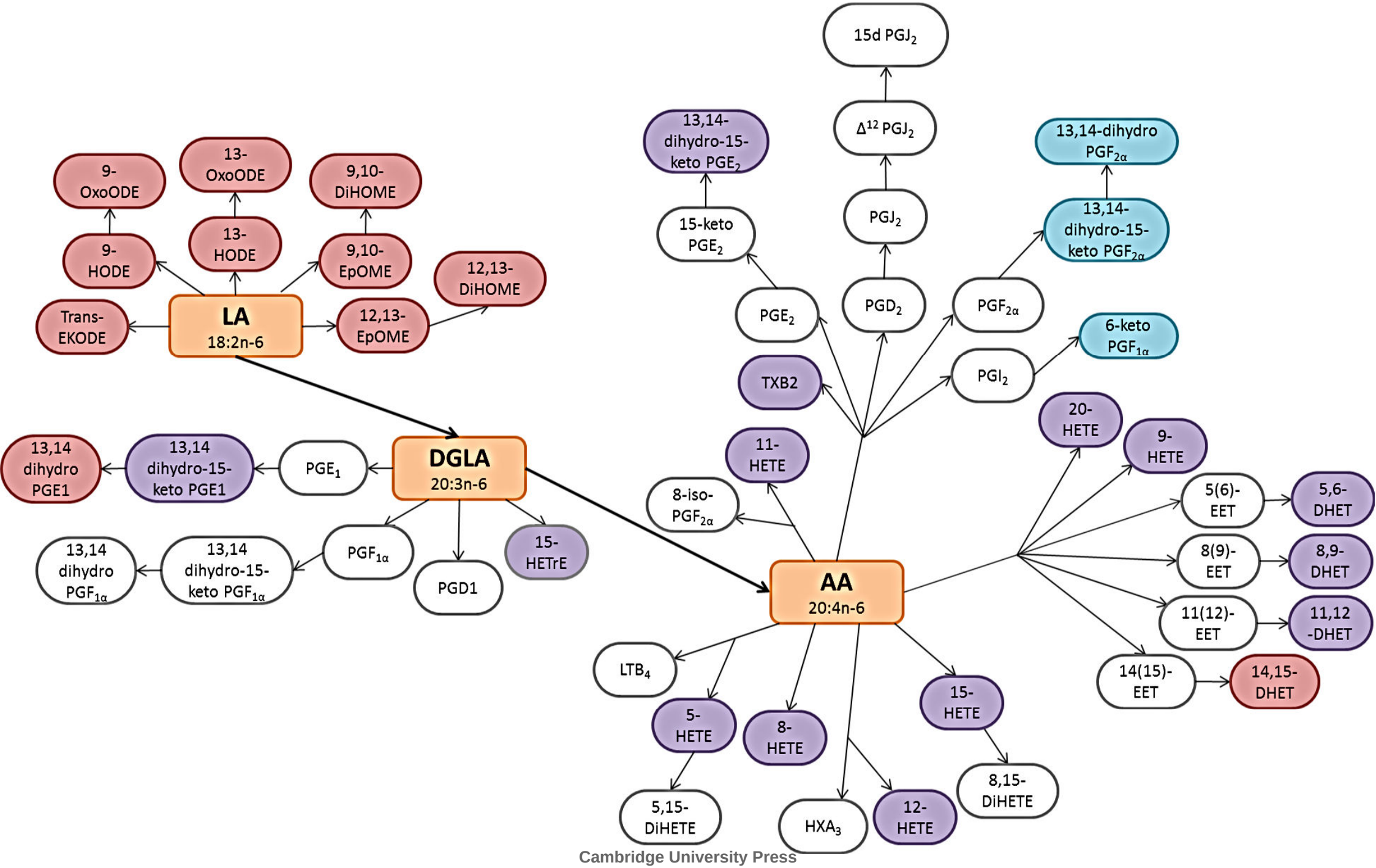
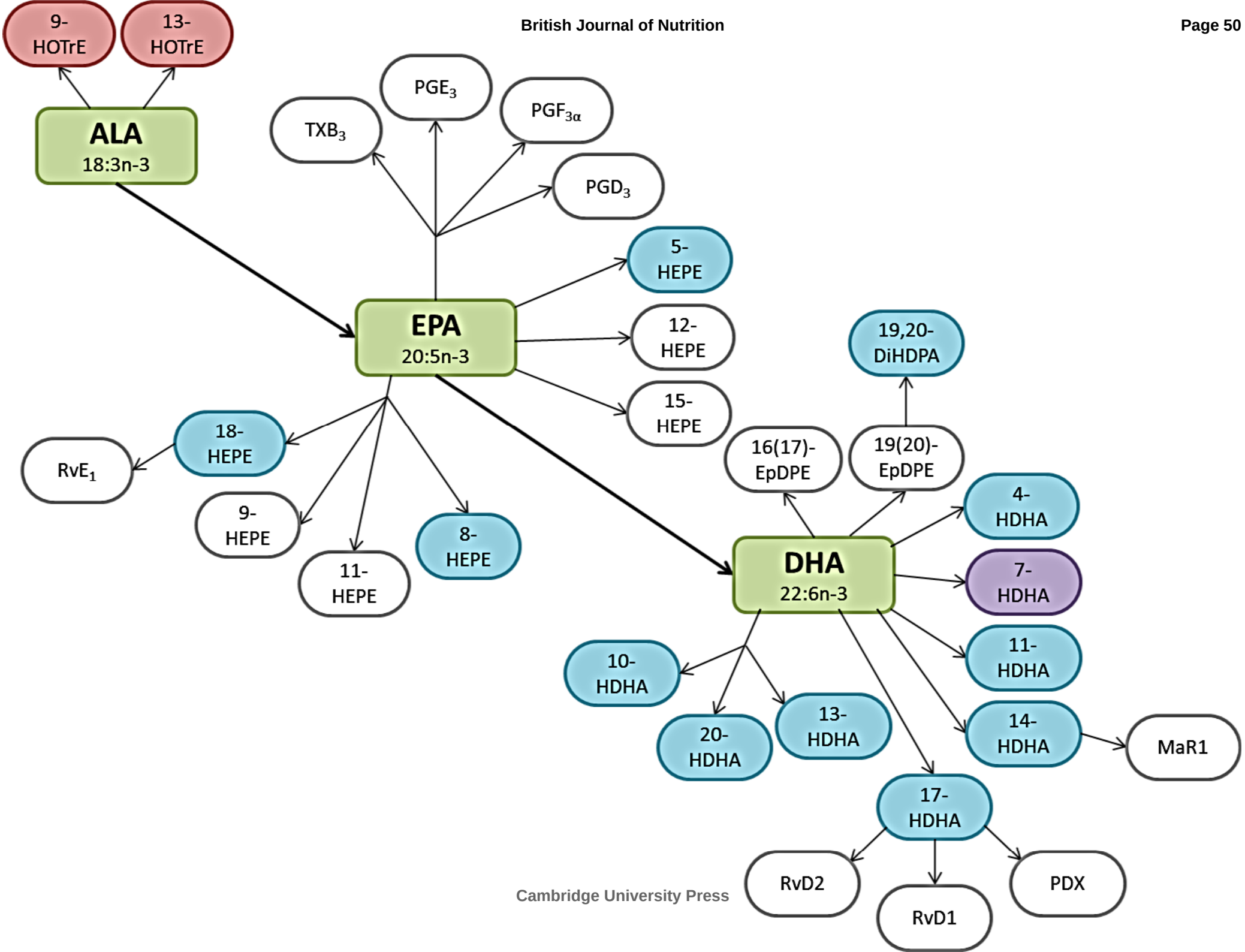



LA
18:2n-6
9-OxoODE
13-OxoODE
9,10-DiHOME
9-HODE
13-HODE
9,10-EpOME
12,13-DiHOME
Trans-EKODE
12,13-EpOME
DGLA
20:3n-6
13,14 dihydro PGE1
13,14 dihydro-15-keto PGE1
PGE1
13,14 dihydro PGF1α
13,14 dihydro-15-keto PGF1α
PGF1α
PGD1
15-HETrE
AA
20:4n-6
15d PGJ2
Δ12 PGJ2
13,14-dihydro-15-keto PGE2
PGJ2
13,14-dihydro PGF2α
15-keto PGE2
13,14-dihydro-15-keto PGF2α
PGE2
PGD2
PGF2α
6-keto PGF1α
TXB2
PGI2
20-HETE
11-HETE
9-HETE
8-iso-PGF2α
5(6)-EET
5,6-DHET
8(9)-EET
8,9-DHET
11(12)-EET
11,12-DHET
14(15)-EET
14,15-DHET
LTB4
5-HETE
8-HETE
15-HETE
8,15-DiHETE
5,15-DiHETE
HXA3
12-HETE


9-HOTrE
13-HOTrE
ALA
18:3n-3
PGE3
TXB3
PGF3α
PGD3
5-HEPE
EPA
20:5n-3
12-HEPE
15-HEPE
18-HEPE
RvE1
9-HEPE
11-HEPE
8-HEPE
19,20-DiHDPA
16(17)-EpDPE
19(20)-EpDPE
4-HDHA
DHA
22:6n-3
7-HDHA
11-HDHA
10-HDHA
13-HDHA
20-HDHA
14-HDHA
MaR1
17-HDHA
RvD2
RvD1
PDX